

# **FibreFix Tutorial 4:**

## **Time-resolved Low-angle X-Ray Diffraction from Muscle**

### **1. Introduction**

Time-resolved X-ray diffraction has applications in many areas of science and technology from probing phase transitions and lattice dynamics in solids to chemical reactions and structural changes in proteins. In many of these situations the samples being investigated are non-crystalline but nevertheless give rise to characteristic diffraction patterns. The CCP13 analysis programs, now integrated in the FibreFix package, have been designed in particular with fibrous and polymer samples in mind, to provide tools for processing fibre X-ray diffraction patterns of all kinds.

X-ray diffraction from muscle is an important example of where time-resolved data are particularly useful for following changes in protein conformation. Additionally, the two main proteins involved in muscle contraction, actin and myosin, both form long ordered filaments within muscle cells and therefore produce very characteristic fibre diffraction patterns. This tutorial demonstrates how time-resolved X-ray diffraction patterns from actively contracting muscles may be processed using the CCP13 FibreFix program. In this example, X-ray data from bony fish skeletal muscle is used, but these techniques can equally be applied to X-ray data from any other type of muscle, and more generally to many other types of time-resolved X-ray data.

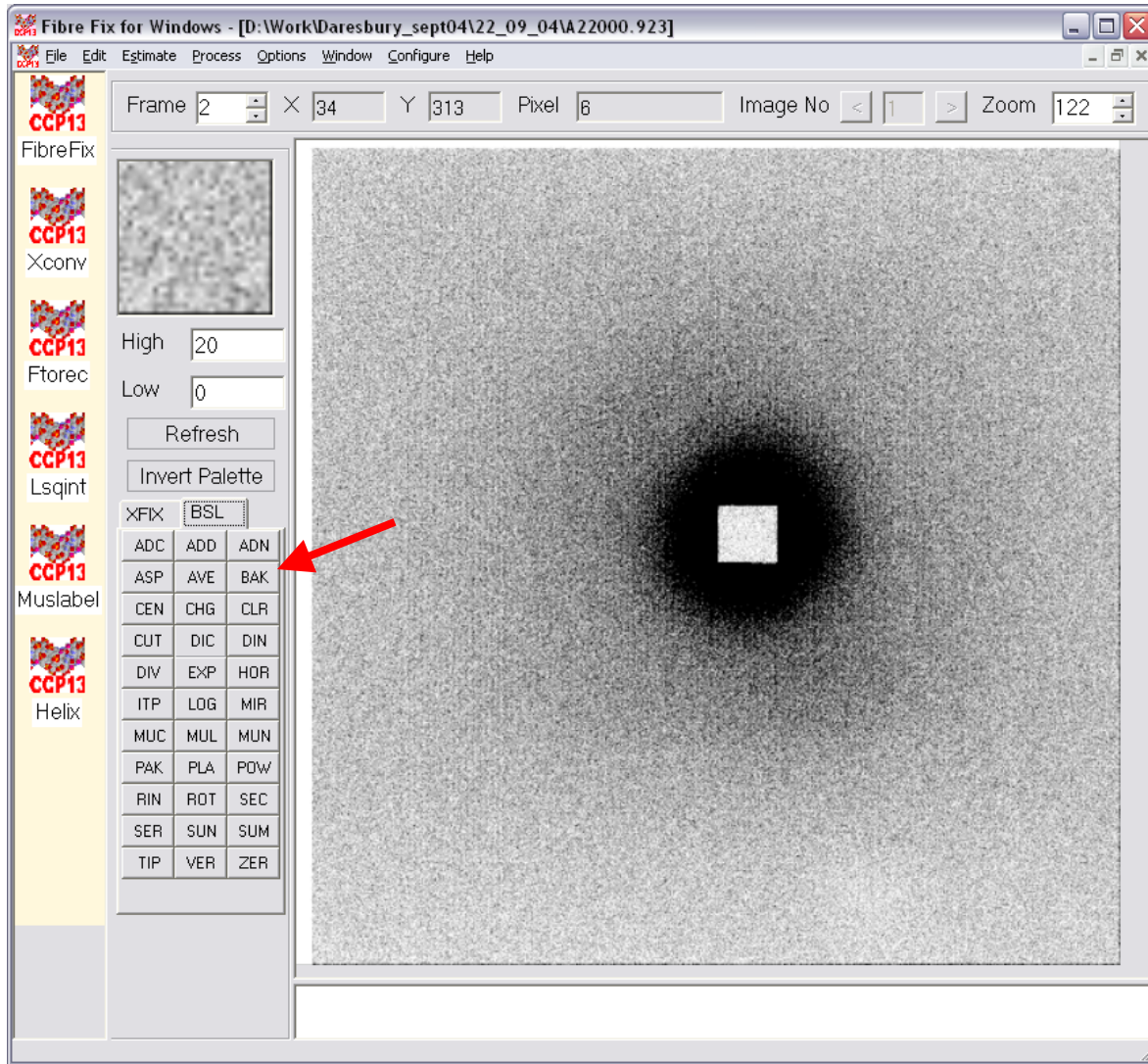
### **2. Muscle X-ray Data Analysis using FibreFix**

#### **2.1 Blank Cell Subtraction and Timecourse Normalisation**

To carry out X-ray experiments on actively contracting muscles, the specimen is very often excised from the animal and mounted in an X-ray cell which contains saline solution to keep the muscle alive and functioning, electrodes to stimulate the muscle to contract, and a tension transducer to measure the tension produced by the muscle during contraction. The whole X-ray cell is placed on the X-ray beam line. It has two very thin windows on either side of the muscle positioned to transmit the X-ray beam pass with minimal attenuation.

To remove any artefacts introduced into the X-ray diffraction patterns by the X-ray cell itself, it is usual for a pattern from the empty cell, without the muscle but containing the saline solution, to be recorded. This pattern can then be removed from the muscle X-ray patterns when the data are analysed. An example of the pattern from a blank cell is shown in Figure 1 below.

One of the main goals of analysing time-resolved X-ray diffraction patterns is to create plots of the changes in the intensities of various reflections during the course of the experiment. The count recorded by the X-ray detector for each pixel is proportional to the intensity of the X-rays incident on that pixel. However, things other than changes in the specimen structure can affect the intensity of the reflections in the pattern. The X-ray flux from the synchrotron itself is not constant, reducing with time since the last beam injection. This can be measured, for example, with an ionisation chamber placed before the sample on the beamline, giving a total ionisation count for each frame of the timecourse, which can usually be saved by the beamline acquisition system in a calibration file. The calibration file can then be used to normalise the data and eliminate the effects of varying incident X-ray flux.



**Figure 1.** X-ray pattern from a 60s exposure of a blank X-ray cell, containing saline solution but no muscle, displayed from 0-20 counts.

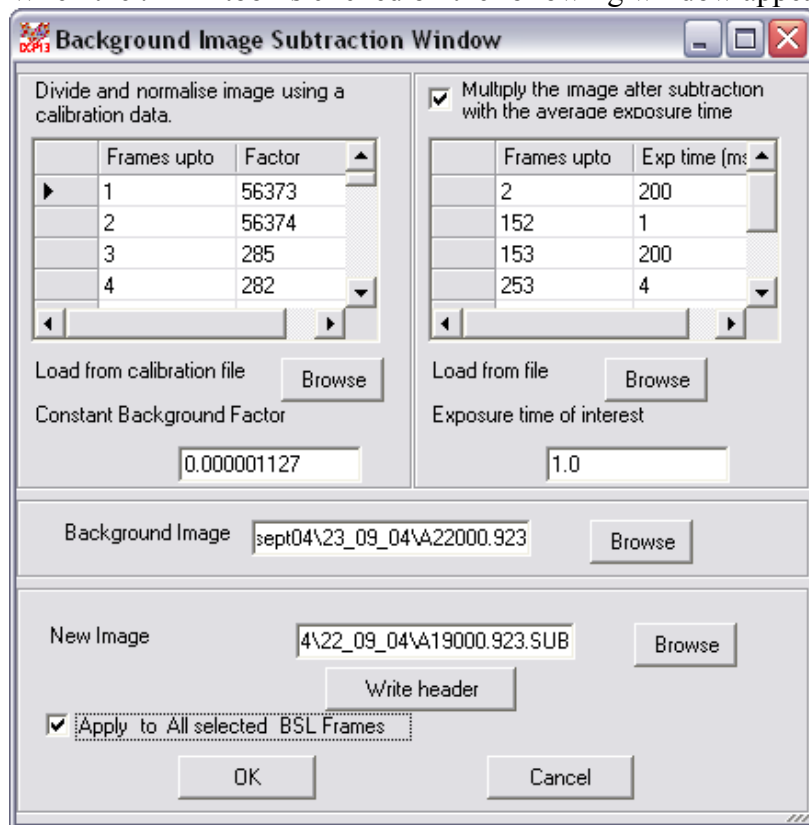
In FibreFix this process has been combined with the removal of the blank cell pattern from the timecourse, using the BAK tool in the BSL tab (see arrow in Figure 1). The equation which this tool applies to the timecourse is:

$$\text{Normalised Timecourse} = \left( \frac{\text{Original Timecourse}}{\text{Blank Cell Removed}} - \text{CBF} \times \text{Blank Cell} \right) \times \text{Average Ionisation Count for 1 Frame}$$

Where *CBF* is the constant background factor.

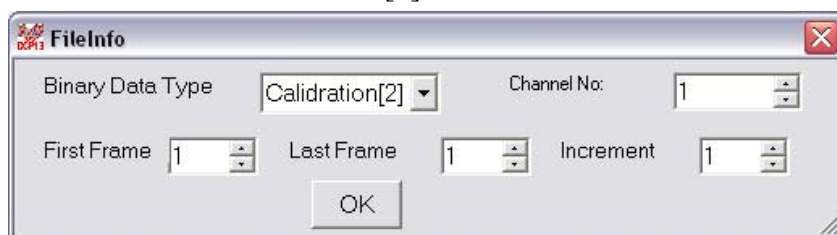
The ionisation chamber data normalises each frame in the X-ray timecourse with respect to ionisation count and time, because the total number of ionisation counts for each frame is recorded – and this not only depends on the X-ray flux but also the exposure time of the frame.

When the .BAK tool is clicked on the following window appears:



**Figure 2. BAK tool dialogue window.**

The calibration file may contain data from more than one acquisition channel in the beamline system. The 'Channel No' field allows the correct channel to be selected. The first and last frame fields should be left as default: 1.



**Figure 3, Calibration file and channel selection window**

When the OK button is clicked, the ionisation chamber data are loaded into the top left hand table in the BAK tool window (Figure 2), where it can be examined to check that it is correct.

If the calibration data are in a text file rather than a beamline acquisition file, the file should be selected in the file manager and the data should load into the top lefthand table in the BAK tool window.

The top righthand section of the window in Figure 2 allows the pixel counts in the pattern to be converted back to real values. Having normalised the timecourse using the ionisation chamber data, the counts for each pixel in each frame are no longer real count rates – all the values are less than one. To correct this, the frames must be multiplied by the average count rate for the frames which are being analysed. In this example the frame exposure times are: 2 x 200ms, 150 x 1ms, 1 x 200ms, 100 x 4ms, 1 x 200ms and 1 x 3700ms. The rising phase of the contraction occurs during the 150x1ms frames and this is the part of the contraction which is being investigated so all the frames in the timecourse will be multiplied by the average count rate for a 1ms frame. If the relaxation

Click the browse button in the top left hand section, to open a file manager window allowing the file containing the ionisation chamber calibration data to be opened. If the data have been saved on the beamline acquisition system, the BSL header file corresponding to pattern being analysed should be selected from the file manager. If the data are just in a text file, this file should be selected.

If a header file has been selected another window appears on top of this one, see Figure 3. The 'Binary Data Type' field allows the user to choose which of the three data files to extract the ionisation chamber data from: SAXS [1], Calidration [2], WAXS [3]. The beamline acquisition system usually stores the data collected in the calidration [2] file.

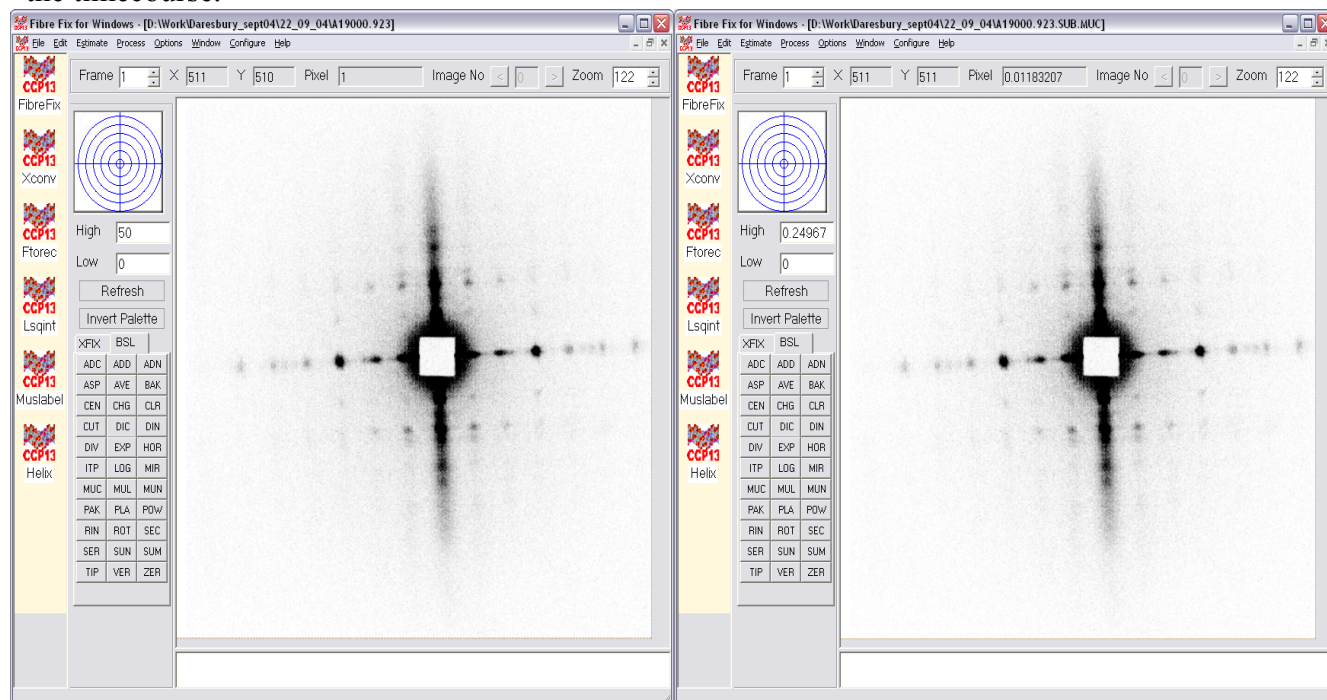
phase, which occurs during the 100 x 4ms frames, was being investigated then all the frames would be multiplied by the average count rate for a 4ms frame so that these frames had real count rates. It must be remembered therefore that the other frames of exposure times different to the length being studied will not contain realistic count rates.

To allow the counts for all the pixels to be converted back to real count values in the frames of interest, the exposure times for all the frames need to be entered into the table in the top right hand side section. Alternatively, if these values are the same for many different files, they can be saved in a text file and loaded using the browse button, as for the ionisation chamber data, thus making the process quicker. The exposure length of the frames being studied should then be entered into the 'Exposure time of interest' box; in this case the 1ms frames are being studied.

The blank cell pattern can now be entered in the middle section of the BAK tool window in the Background Image field (see Figure 2). Again the browse button can be clicked and the header file for the blank cell selected from the file manager window.

The user now enters the Constant Background Factor, which allows scaling of the blank cell pattern. The blank cell pattern usually has a much longer exposure time than the timecourse and so needs to have the pixel counts reduced to the level of the timecourse. This value can be varied by the user until a satisfactory result has been achieved. Or, since the timecourse has been normalised with respect to total ionisation count, the reciprocal of the ionisation chamber reading for the blank cell can be used. This can easily be found by loading the blank cell calibration file into the table above prior to loading the timecourse calibration file. The blank cell is then also normalised with respect to exposure time and X-ray flux.

Finally because the analysis is being carried out for a timecourse of many frames the 'Apply to All selected BSL Frames' check box should be ticked so that this process is carried out for all frames in the timecourse.



**Figure 4: Left - Pattern before blank cell subtraction and normalisation. Right - Pattern after blank cell subtraction and normalisation**

## 2.2 Checking for Rotation Within the Timecourse

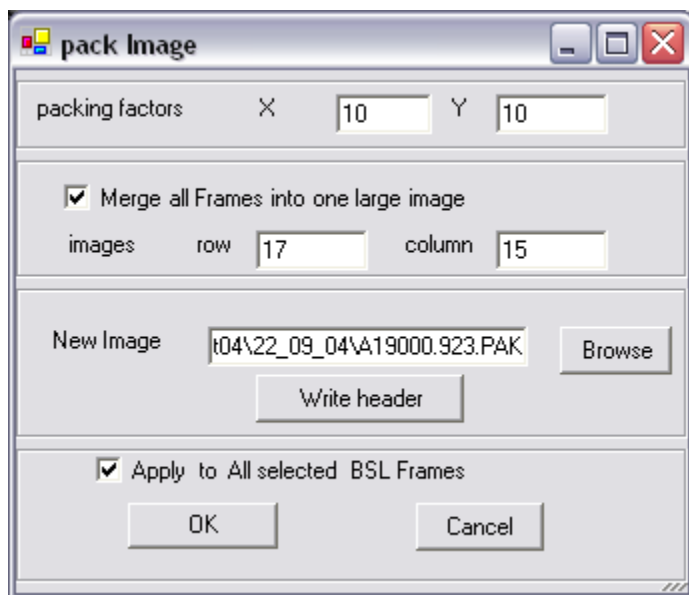


Figure 5: PAK tool dialogue window

large image' box should be checked and the number of rows multiplied by the number of columns should be equal to or greater than the total number of frames so that all fit on the same image. The output file is as shown in Figure 8.

Another useful method of checking for rotation is the BSL tool PLA, see the red arrow in Figure 8. The tool can only be applied to files with multiple frames and it displays each frame in sequence at a speed defined by the user. Thus, if the frames are displayed at a reasonable speed, any variation in rotation is quite easy to spot.

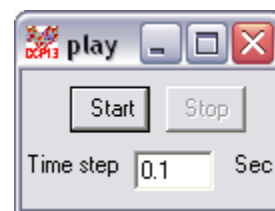
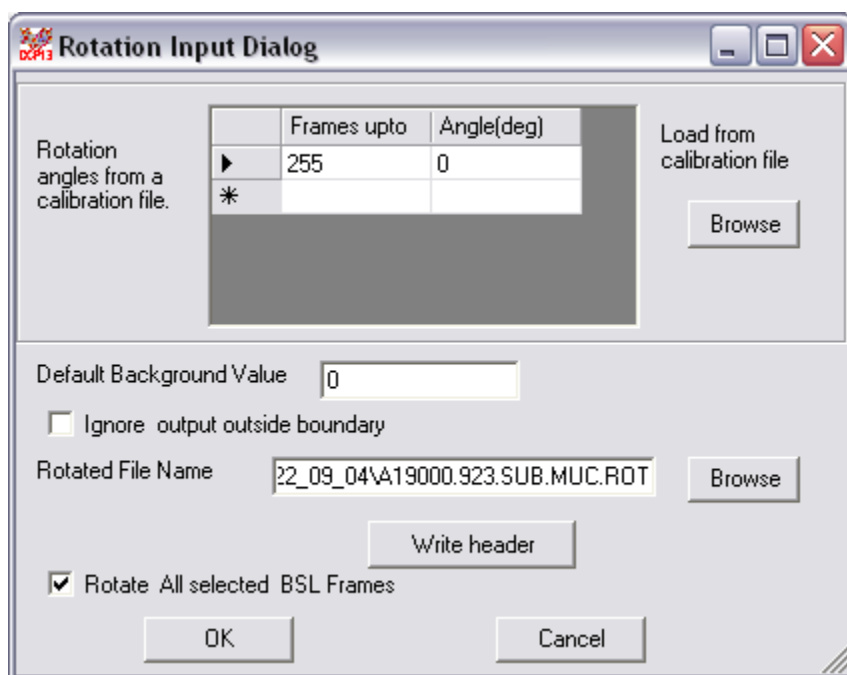


Figure 6: PLA tool dialogue window



If the timecourse is found to rotate, the BSL ROT tool (arrowed in Figure 8) can be used to correct this. The window which appears when this tool is selected is as shown in Figure 7.

By using the table in the top section of the window the rotations to apply to different frames can be input, allowing all the frames to be aligned with the meridian vertical and the equator horizontal.

Figure 7: ROT tool dialogue window



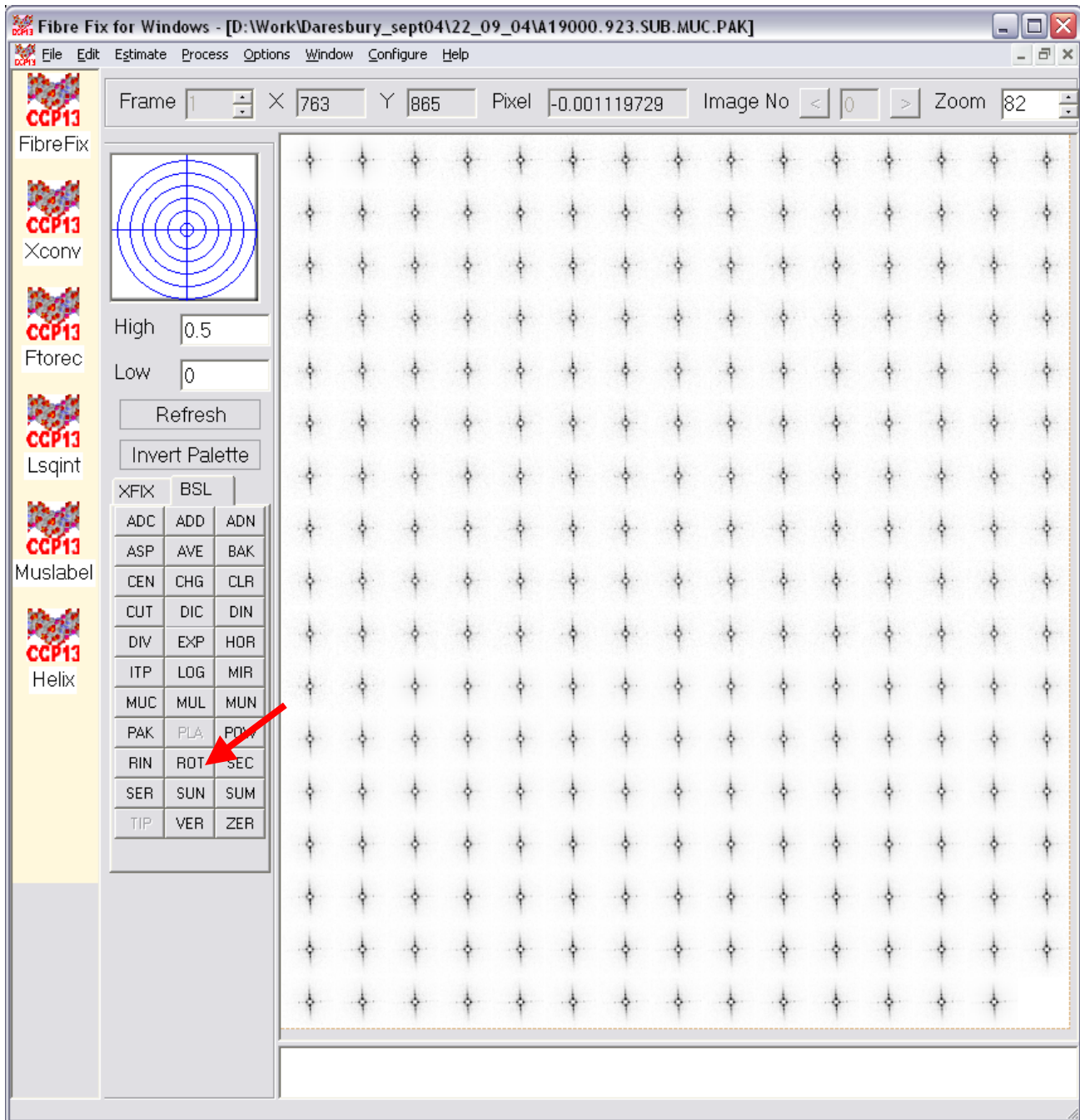


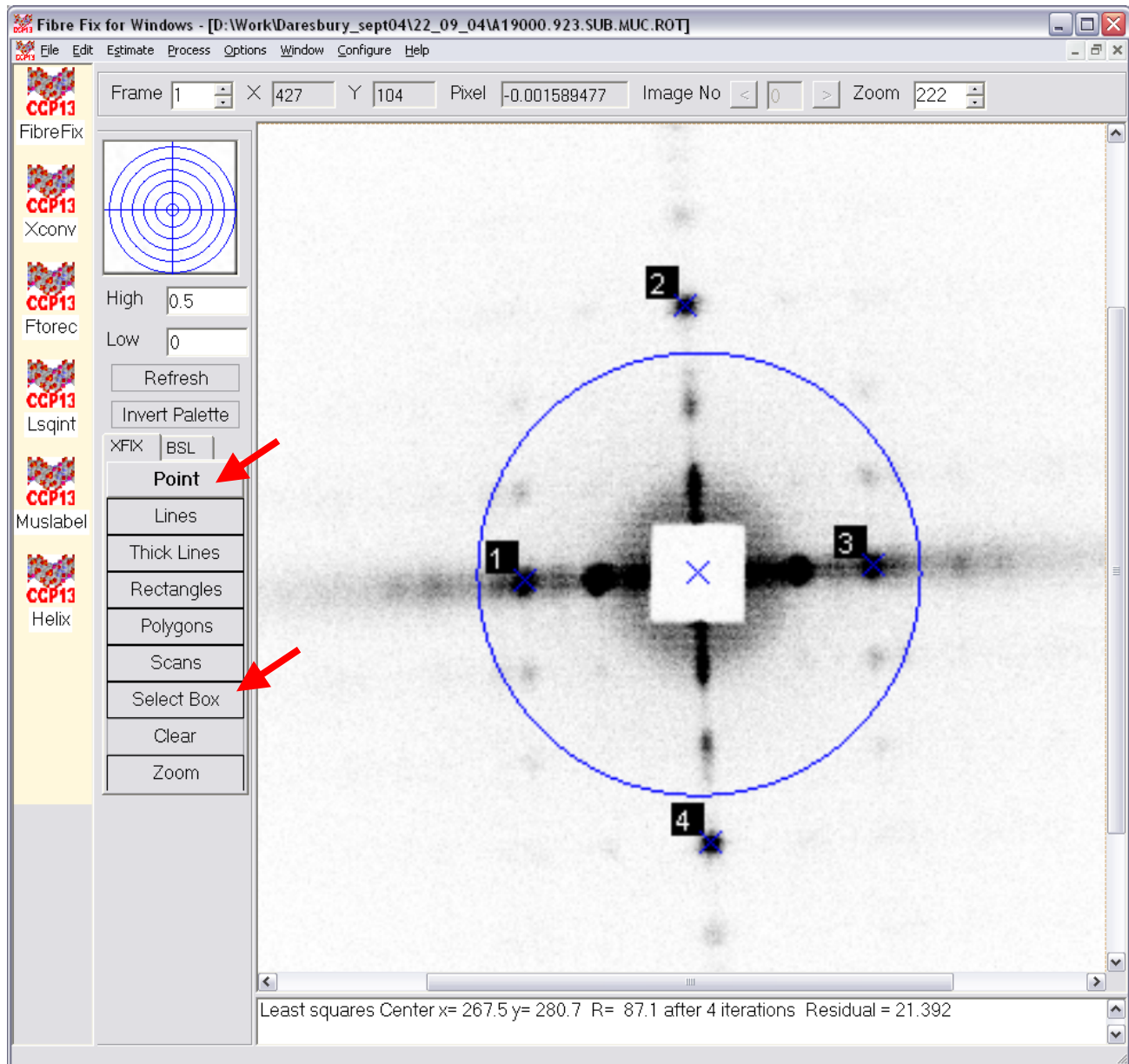
Figure 8: PAK tool output

## 2.3 Finding the Centre, Rotation and Tilt of the Patterns

Before any data can be input into the ROT tool, (i) the centre, (ii) the angle over which to rotate the pattern to align it and (iii) the tilt of the specimen need to be estimated, using reflections in the pattern, and refined. The pattern being used in this case was recorded with the equator closer to vertical than horizontal, so the first thing to do is perform a set rotation of  $90^\circ$  to all of the frames in the timecourse using the ROT tool, so the pattern conforms to the standard: equator horizontal, meridian vertical.

The centre of the pattern then needs to be estimated. Two sets of two symmetric reflections need to be used for centre estimation. The best reflections to use for this in a muscle pattern are the M3

reflections on the meridian and the [1,1] reflections on the equator. These need to be selected using the Point tool in the XFIX tab (see Figure 9), by clicking on the tool and then clicking in the centre of the reflections to place a numbered point there. Once the reflections have been selected then the right mouse button should be clicked and the 'estimate centre' option selected. The program will give a value for the centre and also draw the circle on the image visible in Figure 9. A window will pop up with the centre coordinates.



**Figure 9: Centre estimation**

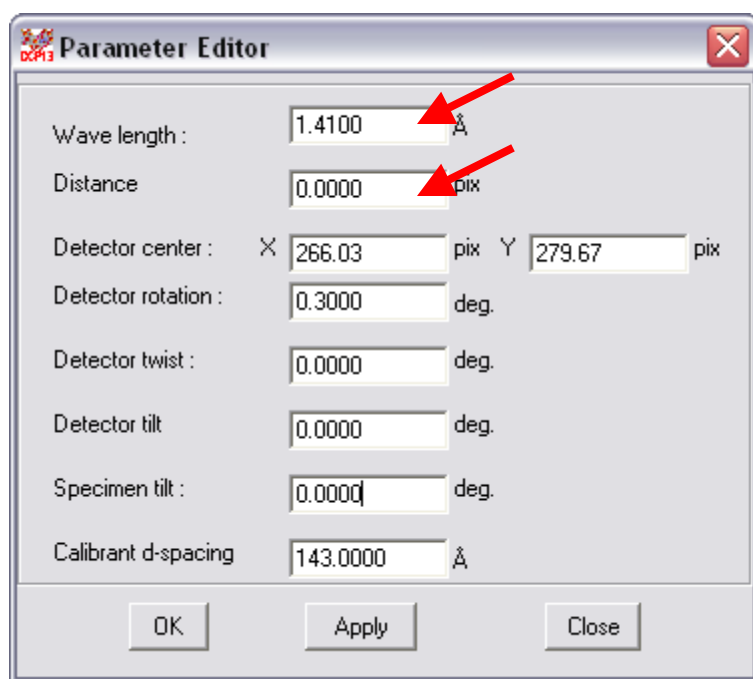
These values have now been saved in the parameters section of the Edit menu.

The rotation can be estimated in a similar way by first clearing the points used for the centre estimation using the clear tool in the XFIX tab and then selecting mirrored reflections on either side of the meridian using the Point tool again. The [1,1] reflections on the equator are good to use in muscle patterns. The meridional reflections can't be used for rotation estimation because the program requires symmetric reflections on either side of the meridian to estimate rotation. This time

the 'estimate rotation' option should be selected from the right mouse button menu and a window will pop up with the estimated value. This is also saved to the parameter section.

To be able to estimate the tilt of the specimen, the program requires the detector to specimen distance in pixels. This also can be calculated by FibreFix using a reflection of known spacing in the pattern. In the case of muscle, the M3 reflection is a good one to use, but other peaks such as calibration peaks from a known substance can also be used. Firstly, the spacing of the known reflection must be entered in Angstroms into the Calibrant d-spacing box in the Parameter Editor (see arrow in Figure 10).

Using the Point tool in the XFIX tab, the known reflection should then have a point placed in its centre and the right mouse button should be clicked. 'Estimate Distance' should be selected from the menu. This will cause a window to pop up with the estimated camera distance, which will also be saved in the parameters section of the Edit menu (see arrow in Figure 10).



The wavelength of the X-rays in Angstrom also needs to be entered into the Wavelength box. To estimate the specimen tilt, two symmetric reflections on either side of the meridian and as far from it as possible above the equator need to be selected using the point tool. In the case of fish muscle, the outer part of the M3 layer-line (e.g at the [1,1] position) can be used here, but if a higher resolution reflection is visible this will give a better result. The 'estimate tilt' option should then be selected from the right mouse click menu. A window will pop up with the estimated specimen tilt and this value will be saved to the parameter section as well.

**Figure 10: Parameter window**

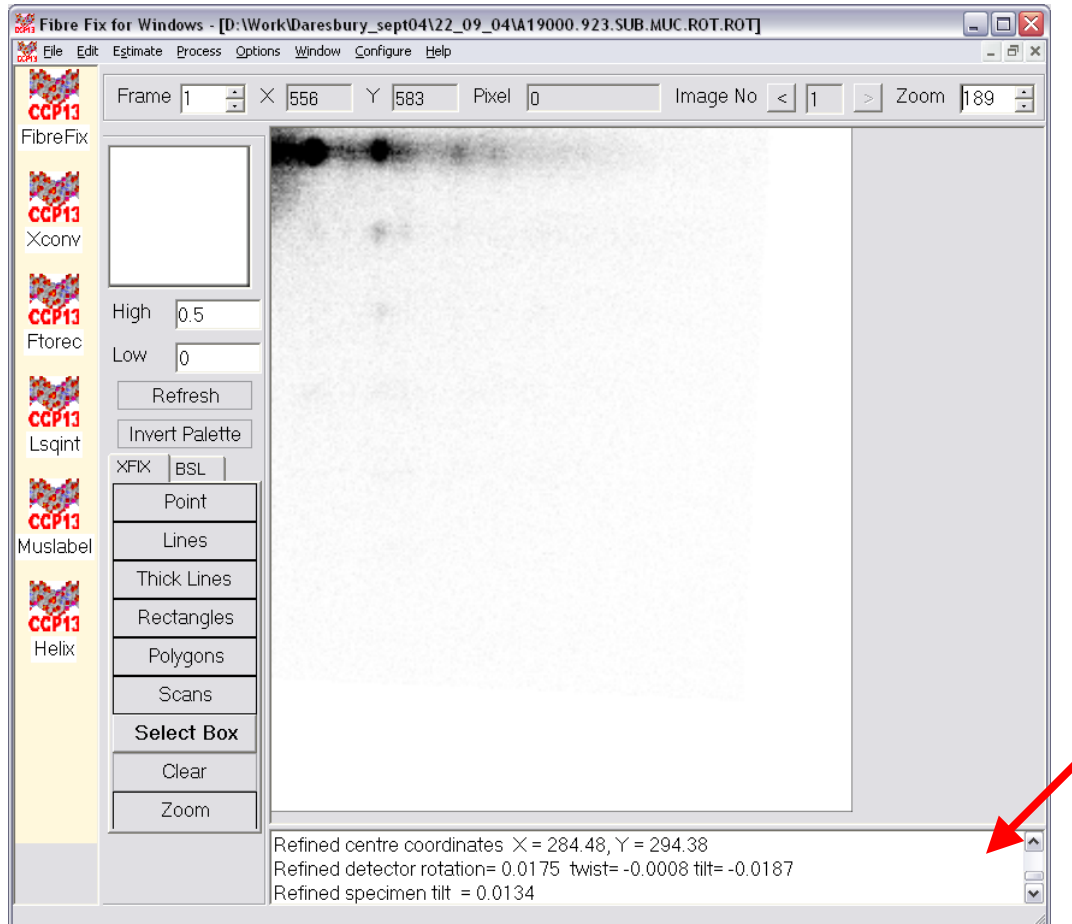
Sometimes, when the actual specimen tilt of the pattern is zero, the tilt estimated by the program becomes very large. In this case it is best to ignore this value and set the specimen tilt to zero in the parameter editor. For these patterns, estimated tilts of up to about 5° are acceptable.

Now that the centre, rotation and tilt of the pattern have been estimated, these parameters can be refined along with the detector twist and detector tilt. Firstly a suitable part of the pattern needs to be selected using the 'Select Box' tool in the XFIX tab (see Figure 9). A part of the pattern without any artificial contrast in it, for example away from the backstop or edges of an attenuation strip, should be selected, making sure there are some prominent reflections in it. For muscles a box encompassing one of the pattern quadrants, excluding the backstop, is often quite successful (see Figure 11).

Once the area has been selected, the 'Refine' option in the 'Process' menu on the toolbar should be selected and a window as in Figure 12 will appear. Initially it usually best to refine all five

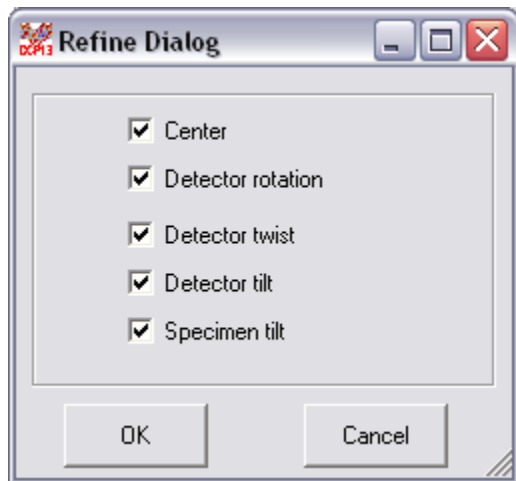


parameters by selecting them in the refine dialogue window. Once the OK button has been pressed, another window will appear whilst the refinement takes place. It can be stopped at any time by pressing the cancel button in this window.



**Figure 11: Example area for use with parameter refinement**

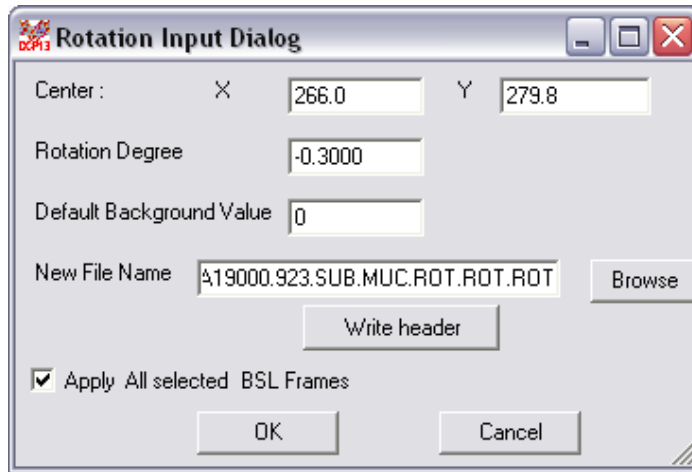
When the refinement has finished, the new parameter values will be displayed in the panel at the bottom of the FibreFix window, see arrow in Figure 11, and another window will appear with the message 'Accept new parameter values'. If yes is chosen, the new values will be saved to the parameter editor (Figure 10), and the next round of refinement will start from these new values. If no is chosen the values won't be saved and the previous set of parameter values will be used for the next refinement.



**Figure 12: Refinement dialogue window**

The process of refinement should be repeated for all the parameters until their values become reasonably constant. However, if one of the parameter values starts to blow up, it may be useful to stop refining this parameter, or to try changing the selected area of the pattern used for refinement to see if this helps.

Once the user is satisfied with the parameter values, the centre and rotation coordinates can be applied to the pattern using the parameter editor opened by clicking 'Parameters' in the Edit menu on the toolbar. The parameter editor window will appear (see Figure 10) and by clicking the 'Apply' button at the bottom another window will appear allowing the rotation and centre to be applied to each frame in the timecourse, see Figure 13.



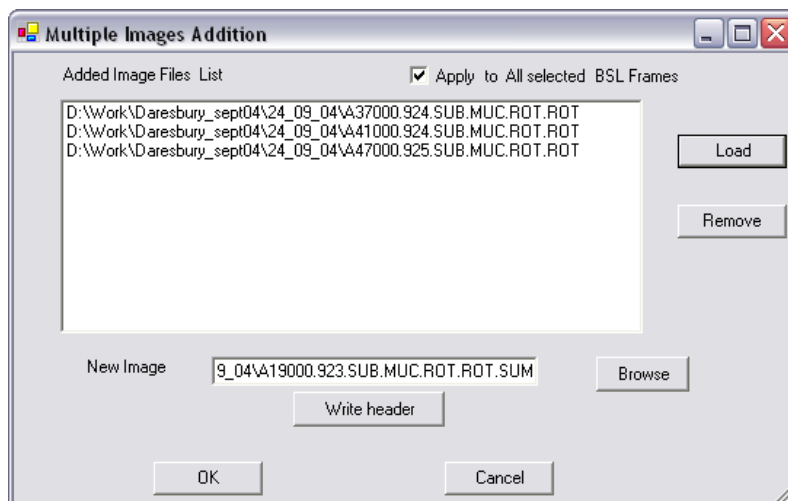
The rotation value will be the opposite sign to the refined value because, if a pattern has a rotation of  $0.3^\circ$ , then it needs to be rotated by  $-0.3^\circ$  to make it straight. The centre and rotation should be applied to all the frames in the timecourse using this tool and then any additional rotation which some individual frames may need can be applied using the ROT tool as described in section 2.2. The extra rotation of these frames can be found by estimating and refining just the rotation for each of the selected frames.

**Figure 13: Apply centre and rotation dialogue box**

In the case of the muscle time series used here it was found that the changes in rotation are generally quite sudden. There was a change from the initial rotation of resting frame one to the rotation of the long active frame at the tension plateau at about frame 15, and then a change back to the initial rotation, or slightly less, around frame 220. Therefore, the longer exposure frames at the start (resting), active plateau and end (resting) of the time series could be used to find the rotations of the shorter frames with the same rotation. This of course will depend on the experimental protocol, exposure times and contraction duration etc, of the time series and should be checked for each time series.

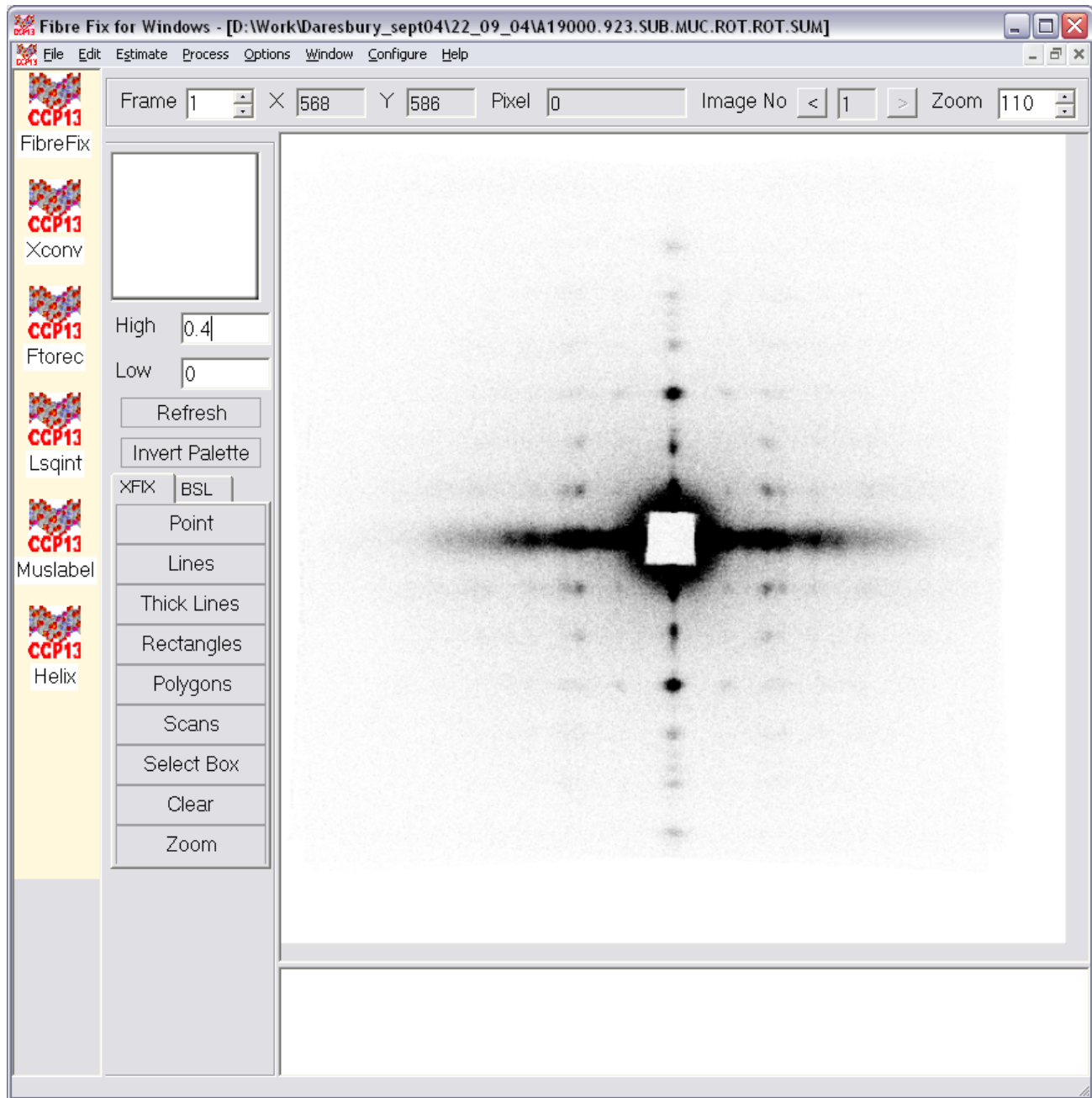
## 2.4 Addition of Time Series

Once all the time series have been centred and aligned with their equators horizontal and meridians vertical, they can then be added together to improve the signal to noise ratio. Each frame in a time series needs to be added to the same frame in all the other time series. This can be done with the BSL tool SUM, which opens the dialogue box:



**Figure 14: SUM tool dialogue box**

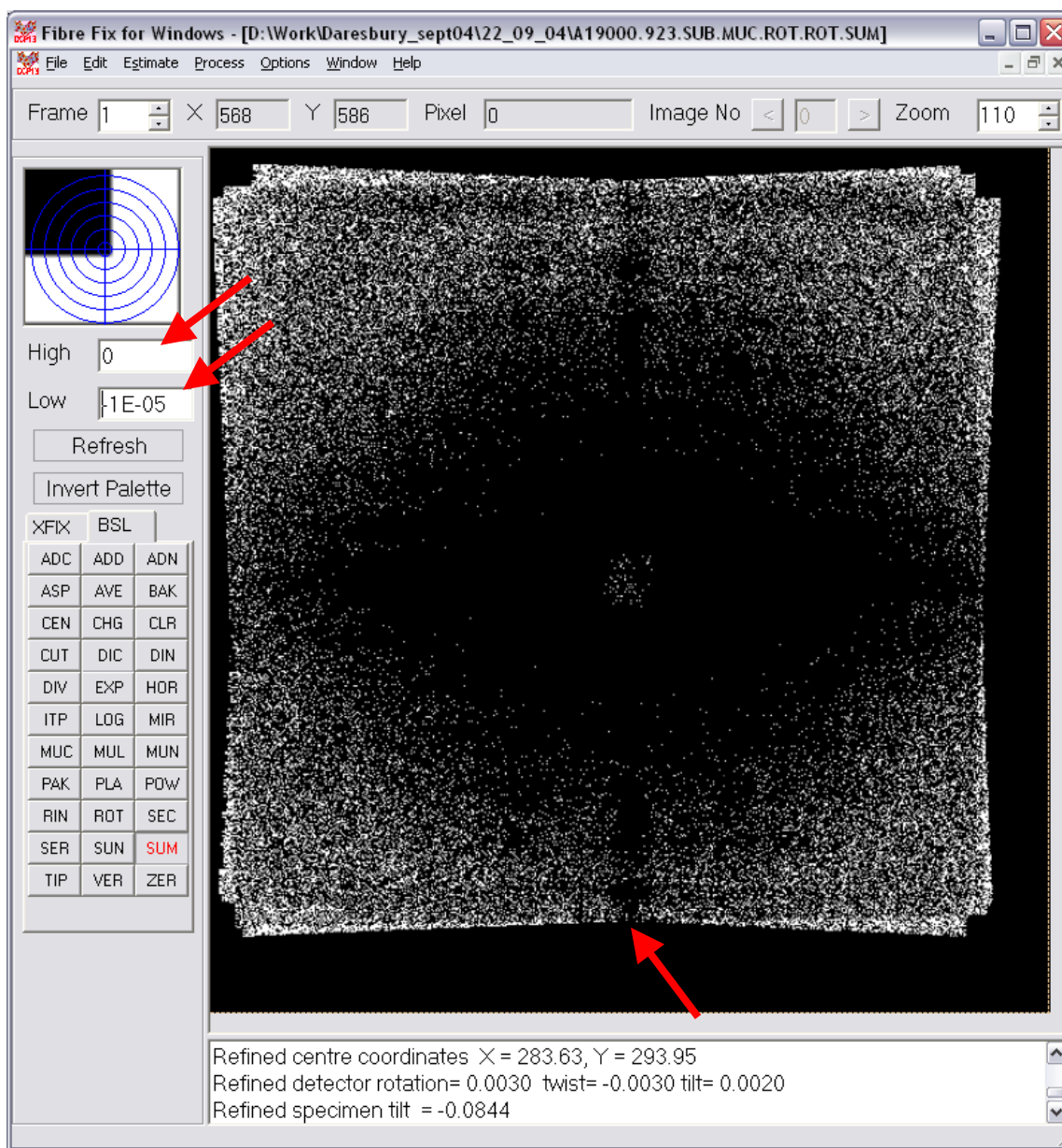
With one of the time series to be added already open, the SUM tool should be selected and the other time series to be added should be loaded into the white box in the window by clicking the Load button and selecting the relevant files. The Apply to All selected BSL Frames box should be checked so that all frames of the same number are added together. When all the time series to be added have been loaded, the OK button should be pressed and the new summed time series will be displayed, as shown in Figure 15:



**Figure 15: Frame 1 of summed time series, 6 time series added**

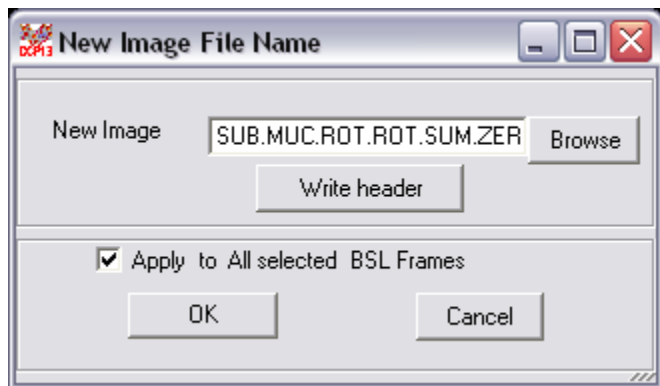
Due to the previous subtraction of the blank cell images from the timecourses, there will inevitably be some pixels with negative counts in the summed time series, because there were some pixels with zero counts, so whatever was taken from them, they will become negative. However, they should be very small negative values. The negative pixels can be displayed in white (all positive pixels black)

by setting the 'High' value (see arrow in Figure 15) to 0 and the 'Low' value to a small negative value, e.g. -1E-05, see Figure 16.



**Figure 16: Negative pixels in frame one of summed time series**

These negative pixels should all be set to zero before the next step in processing. This can be done with the BSL tool ZER, which opens a window as shown in Figure 17:



**Figure 17: Zeroing negative pixels.**

The operation should be applied to all frames and once the OK button has been pushed the new summed time series without negative pixels will be displayed.

## 2.5 Background Fitting and Subtraction

There are three different methods of background fitting available in FibreFix: Roving Window, Circularly Symmetric and Smooth, and additional background fitting and subtraction can be carried out at a later stage in the processing using the program LSQINT. For information on the methods used for the three different types of background fitting in FibreFix, go to Help in the main toolbar and select 'FIBREFIX Help'.

The different background fits can be accessed by going to 'Process' in the main toolbar, moving the mouse over 'Background' and then choosing the method of background fitting required. Getting a good background fit is a process of trial and error and may require using more than one method, and/or the same method more than once with different parameters. The first time a background fit is attempted for a new data set it is useful to try each of the three background fitting methods, using the default parameters, to see which looks most promising and then move on from there.

Often, with time series data, the short frames (in this case the 1ms frames) are difficult to directly fit backgrounds to, due to their low counts and pixels with zero counts. In the case of fish muscle, where the background appears to change rather little, it is often useful to find the background of one of the longer frames and then to subtract this from all the other frames in the time series. No scaling should be needed because all the frames have already been normalised. However, it is likely there will be some negative pixels in the shorter frames for the reasons discussed in section 2.4 and these again must be removed using the ZER tool in the BSL tab, see section 2.4. In the case of muscle timecourses, the long active frame may produce the best background fit. This may be true with fish muscle since the highly sampled myosin layer-lines are much weaker. So in the fish muscle example used here, the background has been fitted to the 200ms long active frame and then subtracted from all the other frames.

The active frame can be separated out from the time series, to allow the background fitting to be applied separately, when the time series is first opened. The dialogue box in Figure 18 appears when the time series is opened and, if the First Frame and Last Frame boxes are set to the active frame number, then only this frame will be opened. It can then be saved separately, using 'Save' from the File menu.

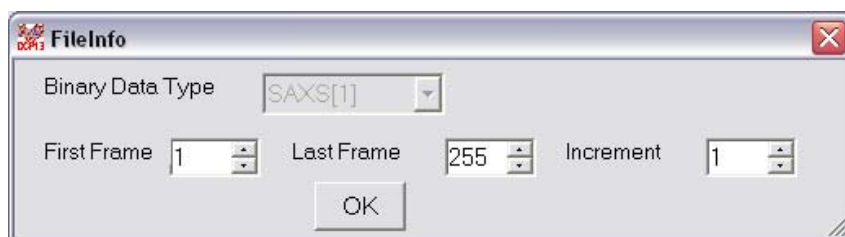


Figure 18: File Info dialogue box

In this example the active frame is fitted firstly with the Smoothed background. When this method is chosen from the process menu, the window shown in Figure 19 appears. The centre of the pattern will now be the centre of the image window as the time series was centred and should automatically be inserted by the program. The pattern limits determine the radius of the pattern to which the background fit is applied. The rest is masked out with a constant value of  $-1E+30$ . In this example the pattern limit was chosen from 0 (the centre of the pattern) to 230, the edge of the pattern in the image window closest to the centre (see arrow in Figure 16).



For the smoothed background fit there is a choice of two smoothing functions which can be used: Gaussian or Box Car. In this case, Gaussian was used with a Full Width Half Max (FWHM) of 20 pixels and 5 cycles of smoothing. The resulting file contains both the background (first frame) and the pattern with the background removed (second frame). If more frames had been present this cycle would have continued through the time series.

**Smoothed background**

Center X: 284.5 pix Y: 293.5 pix

R min R max

Pattern limits: 0 pix 230 pix

Discard values less than: 0

Smoothing function: Gaussian

Box car size X: 0 pix Y: 0 pix

Gaussian FWHM: 20 pix

Number of cycle: 5

☐ Apply smoothing at edge of pattern

Import background: [ ] Browse

Frame number: 1

☐ Merge backgrounds

Smoothing factor: 0.000

Tension factor: 0

Weight of imported background: 0.5

Output filename: JC.ROT.ROT.SUM.BAK Browse

Write header

☒ Apply to All selected BSL Frames

Run Cancel

**Figure 19: Smoothed background fit parameter window**

Figure 20 shows the active pattern, fitted background and active pattern – background. It is obvious that the diffuse scattering at the centre of the pattern has been greatly reduced, but there is evidence of reflections in the fitted background and these need to be removed. To remedy this, background fitting can be applied to the fitted background itself to try and extract the reflection information from it. The new fitted background frame should now contain less reflection information and if the user is satisfied that there is no reflection information left, it can be subtracted from the whole timecourse.

***Background fitting of active pattern => active background + active reflections***

However, the active background still contains some of the reflections from the active pattern so:

$$\text{Background fit of active background} \Rightarrow \text{background(active background)} + \text{reflections(active background)}$$

Therefore, by subtracting background(active background) from the active pattern, we are left with active reflections + reflections(active background) and hopefully no reflection information will be left in the second fitted background. This process may be repeated if there are still reflections visible in this second background fit.

In the present case, another smoothed background fit was applied to the background from the first fit. The same centre and pattern limits were used and Gaussian smoothing with a larger FWHM of 50 and 5 cycles of smoothing was applied. The resulting background(active background), reflections(active background), active reflections and active pattern - background(active background) are shown in Figure 21.

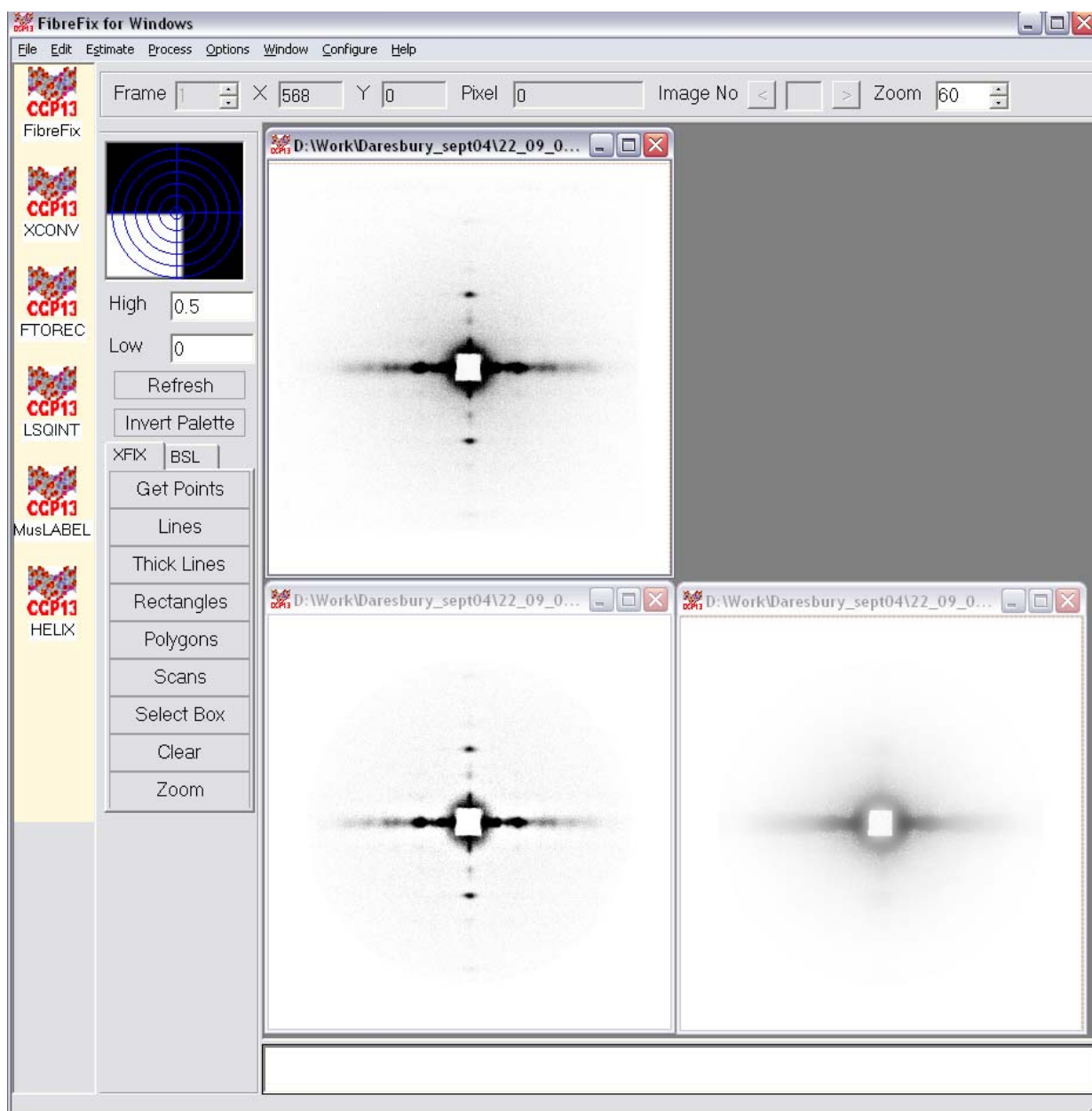
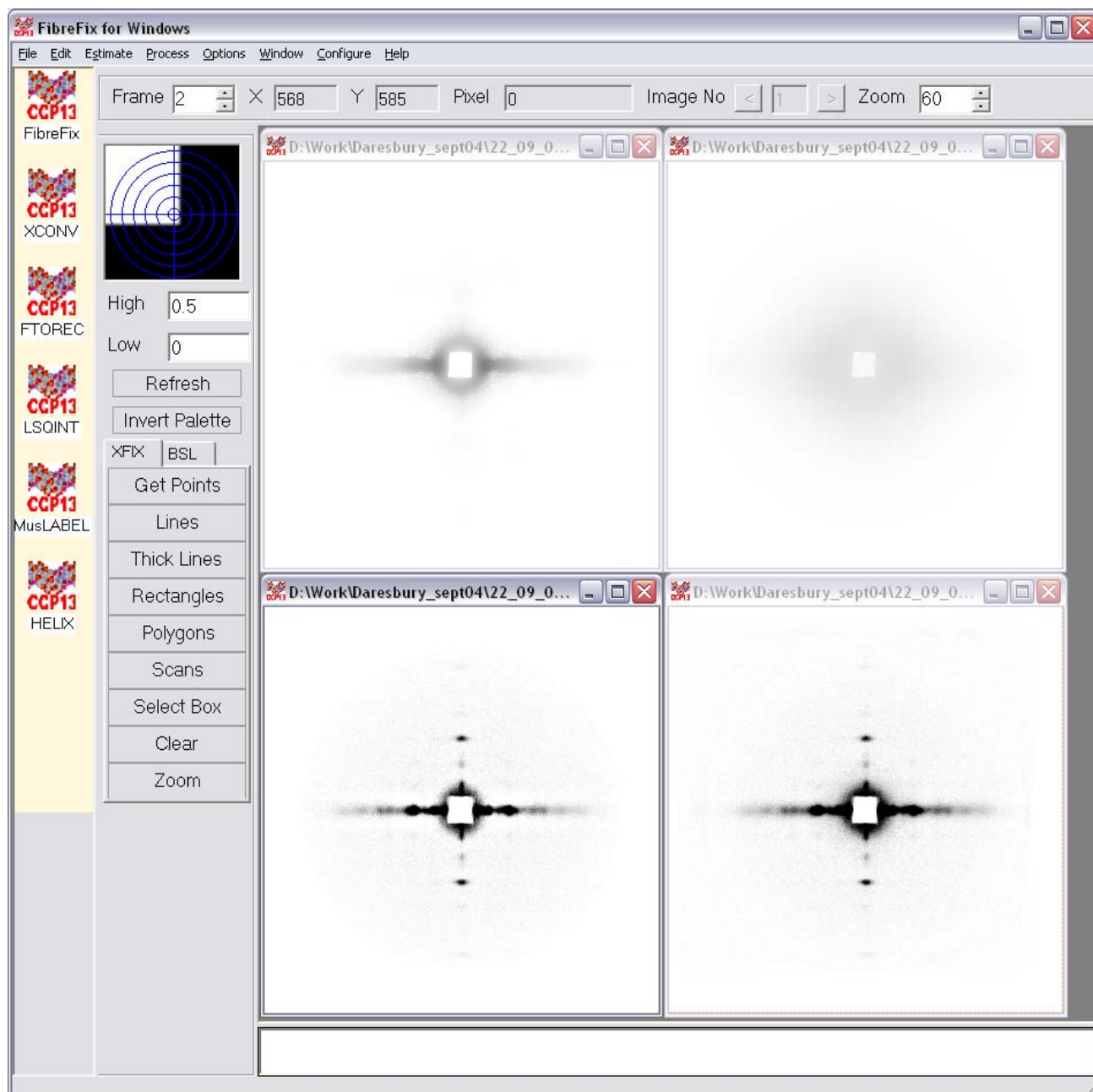


Figure 20: Top left: active pattern before background subtraction; bottom left: active pattern – background; bottom right: background.

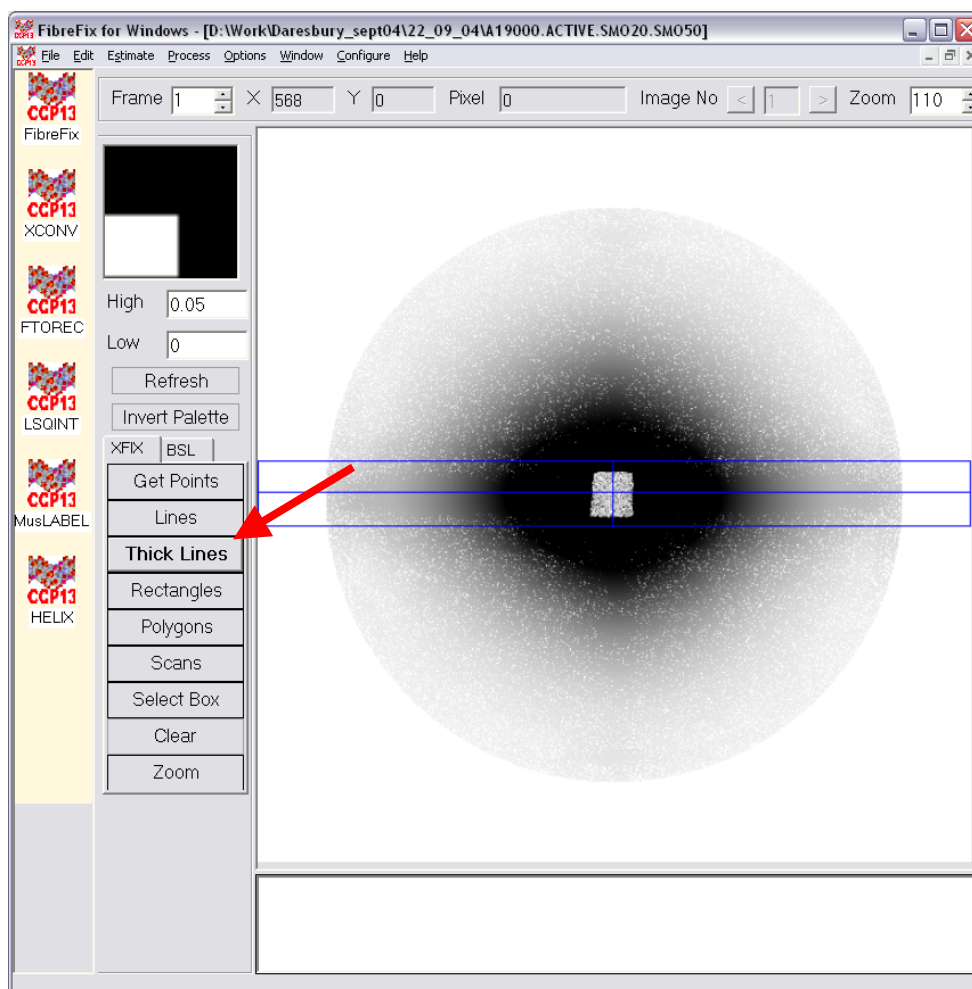
The extent of the reflection information in the fitted background can be checked by taking an intensity profile along the equator, meridian or one of the layer lines to look for reflections. This can be done using the Thick Lines tool in the XFIX tab (see arrows in Figure 22). Firstly a line is drawn along the length of the required profile by clicking on the end points with the mouse. A second line is then drawn in the same manner to specify the width of the profile, and a blue rectangle should appear to denote the area of the profile (see Figure 22). The user should then click the right mouse button to open another window displaying a plot of the intensity along the profile (see Figure 23).



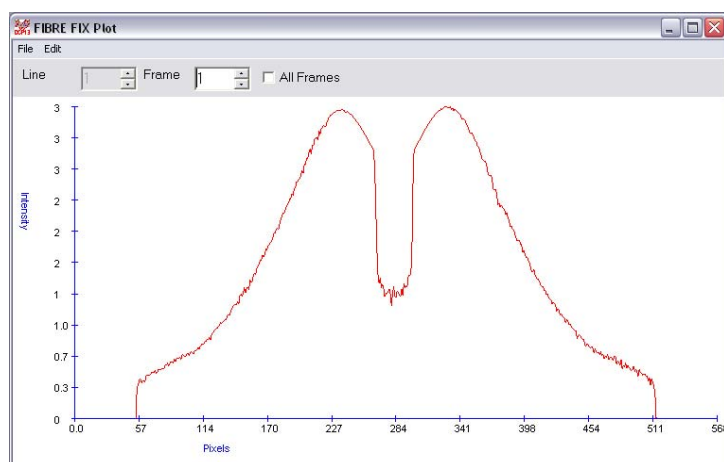
**Figure 21: Top left: reflections(active background); top right: background(active background); bottom left: active reflections; bottom right: active pattern – background(active background).**

Figure 23 shows that the equator of the background fit to the first background has no evidence of any peaks other than the central halo around the backstop. This was also checked for the meridian and

the first layer line and no evidence of other peaks was seen, suggesting that all the reflection information had been removed from the background.



**Figure 22: Background (active background) with profile across the centre selected**



**Figure 23: Thick line profile through the centre of background (active background) as depicted in Figure 21.**

Another important factor to check after background fitting is if there are any negative pixels in the pattern – background or reflection frame. This can be done by adjusting the High and Low values as described above in section 2.4. If many pixels are negative, too much background has been subtracted and the parameters of the fit should be adjusted to get rid of these negatives.

Now that this background fit has been checked and the user is satisfied with it, it can be subtracted from the rest of the timecourse using the BAK tool in the BSL tab. The data in the top 2 panels should be

left with the default values and the background(active background) file should be loaded into the

background image section using the browse button. It is likely from the processing that this file will contain the background(active background) as the first frame and the reflections(active background) as the second frame. Once the OK button has been clicked in the Background Image Subtraction window a Fileinfo window will pop up allowing the user to choose which frames to subtract. The First Frame and Last Frame boxes should be set to the frame number of the background(active background) pattern, which in this example is 1.

To remove more of the central scatter halo around the backstop visible in the active pattern – background(active background) pattern in Figure 21, a circular symmetric background was fitted to the pattern. The window which pops up when the circular symmetric option is chosen from the ‘Background’ option in the ‘Process’ window is shown in Figure 24.

Again the same centre and pattern limits were used as for the other two background fits, and the background was fitted to the active frame once it had had the previous background fit subtracted. The pixel range used was 0 – 10% to make sure the number of negative pixels was minimised and the smoothing factor was set to 0.0001 so that the fitted background was concentrated at the centre of the pattern.

Figure 25 shows the fitted background and the starting pattern compared to the pattern – background. A large amount of the central halo has been removed from the pattern, making the central equator much clearer.

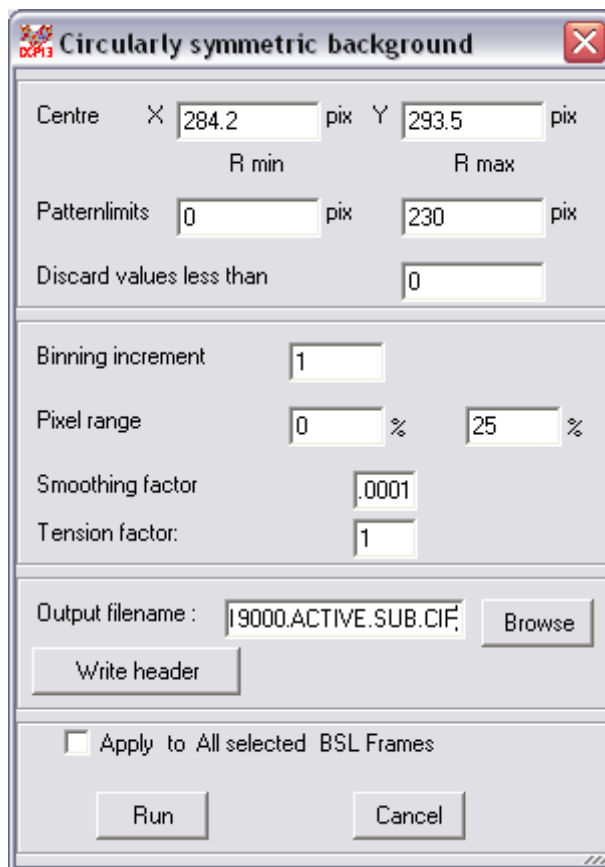


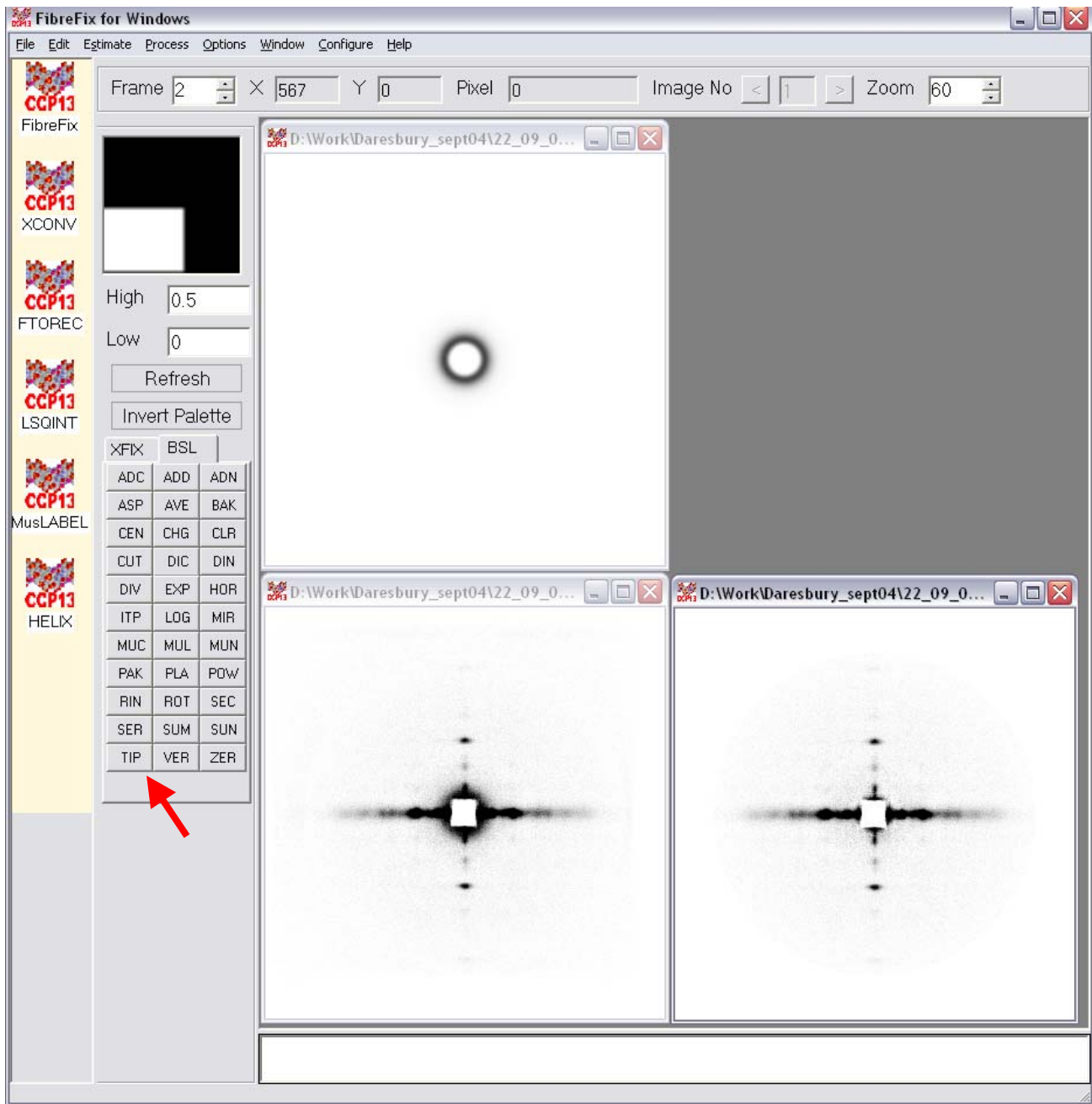
Figure 24: Circularly symmetric background dialogue

This background can now also be subtracted from the rest of the timecourse using the BAK tool in the BSL tab as described above. Also as discussed above any negative pixels can be set to zero.

Further background subtraction can be carried out with these tools but there is also the option of using the program LSQINT, which can be found in the Process menu in the toolbar, to perform more background subtraction. LSQINT has four background fitting options, the roving window and circularly symmetric fits available in the Fibre Fix background fitting together with a global 8 parameter background fit and a flat plane local least-squares fitting. These four options allow further improvement of the background subtraction.

However, the main function of the program LSQINT is to extract reflection intensities from the corrected pattern by fitting and refining the unit cell and profile parameters. The program requires the patterns to be converted to reciprocal space before they can be fitted and so first the data must be put through the program FTOREC to do this.

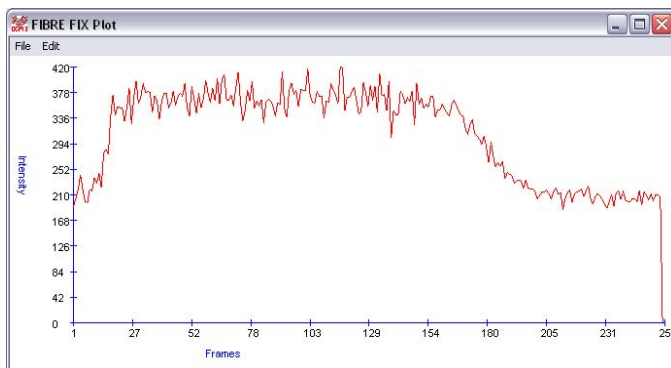




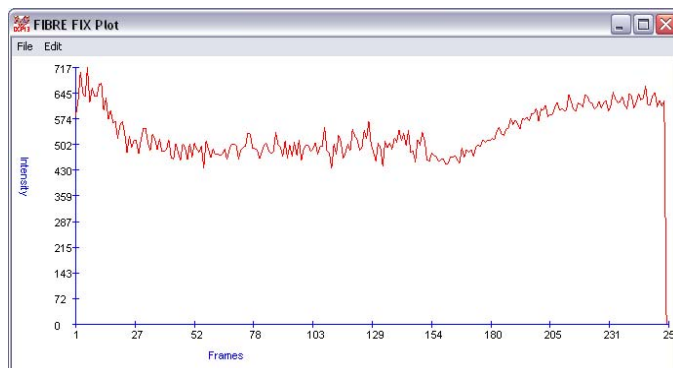
**Figure 25: Top left: circularly symmetric background fit; bottom left: pattern; bottom right: pattern-background.**

## 2.6 Plotting Timecourses of Reflections

Now that a lot of the background has been removed from the patterns in the time series, plots of the intensity timecourses of particular reflections throughout the time series can be made using the new tool TIP in the BSL tab, see arrow in Figure 25. To create an intensity timecourse, the user simply selects the TIP tool and then using the left mouse button, draws a rectangle around the reflection of interest. The right mouse button should then be clicked and a window will pop up with the plot of the total intensity contained in the rectangle against the frame number. In this example, the timecourses of the [1,0] and [1,1] equatorial reflections have been plotted, showing their characteristic variation in intensity as the muscle contracts.



**Figure 26a: Variation in intensity of the [1,1] reflection with contraction**



**Figure 26b: Variation in intensity of the [1,0] reflection with contraction**

## 2.7 Converting patterns to reciprocal space – FTOREC

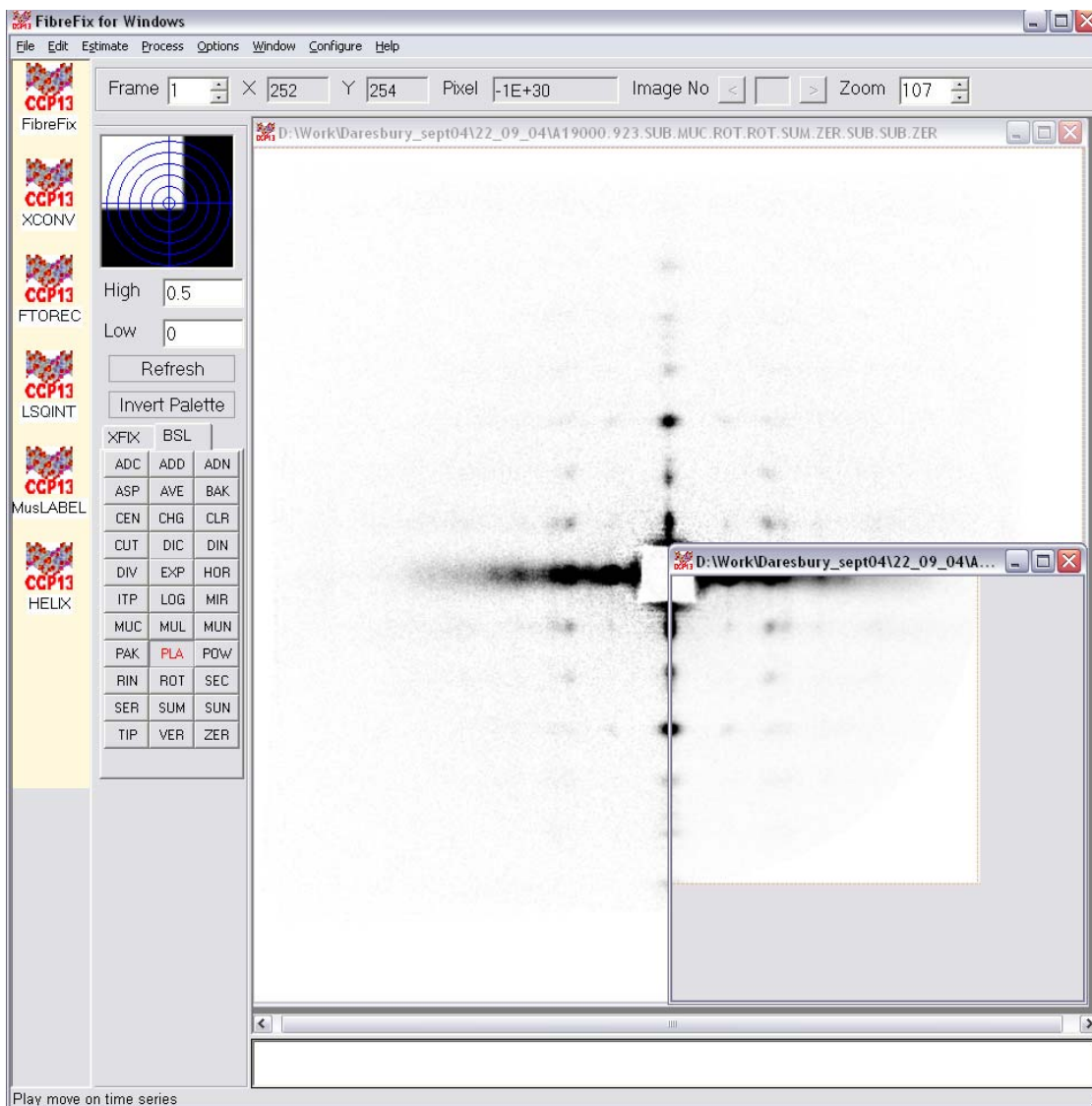
FTOREC can be accessed within the FibreFix program by clicking on the Process drop-down menu in the main toolbar. When FTOREC is selected the window shown below appears.

**Figure 27: FTOREC control window**

The values in the XFIX Parameters section should have been carried forward from the earlier analysis. The user can choose whether a file showing the standard deviations between the four quadrants of the pattern is also produced by checking the box next to 'Standard deviation Output FileName'. This file can often help in assessing whether the parameters used were correct, for

example: if the reflections in different quadrants do not appear to be overlapping properly then the Standard Deviation image shows this more clearly than the output file. This can be remedied by changing the Specimen Tilt or Rotation until the reflections merge.

The values in all the other sections of the window can be left as their defaults, except the 'Dmax' value, see arrow, which needs to be calculated for the maximum resolution of the pattern. In this example the M6 meridional reflection is the furthest out reflection visible. This reflection occurs at  $1/72\text{\AA}^{-1}$  which equals  $0.014\text{\AA}^{-1}$ . Once the user is satisfied with all the parameters, the Run button should be clicked to start the calculation of the transformed pattern. This process again can be applied to all the frames in the timecourse at once. Figure 28 shows the transformed pattern compared to the original pattern for the first frame in the timecourse, a 200ms relaxed frame.



**Figure 28: Original pattern with FTOREC output file of transformed pattern in one quadrant.**

---

**Tutorial written by:**

**Felicity Eakins & John Squire**

**May 19<sup>th</sup>, 2005**